DRAFT

Working Group 7. Genetic Liver Disease

Introduction & Background

Genetic liver diseases include hereditary hemochromatosis, Wilson disease, the porphyrias, cystic fibrosis, polycystic liver disease, alpha-1-antitrypsin deficiency, hereditary tyrosinemia, Alagille syndrome, and several neonatal cholestatic syndromes and inherited diseases of metabolism. Many of these diseases present in childhood and are discussed in other chapters of this Action Plan. This chapter focuses on three genetic liver diseases that present in adolescence or adulthood—hemochromatosis, Wilson disease, and the porphyrias.

Hemochromatosis is probably the most common inherited disorder among Caucasians, affecting ~ 1 in 200 individuals. This disease of iron metabolism is marked by excessive iron absorption and eventual accumulation of toxic levels in the liver, heart, pancreas, joints, and other organs. High iron levels in the tissues are generally not reached until the 4^{th} or 5^{th} decade of life, at which point signs of cirrhosis, heart disease, endocrine failure, or liver cancer may arise. Once the diagnosis is made, hemochromatosis is treated with therapeutic phlebotomy until total body iron stores are reduced to normal. The availability of this safe and effective therapy for a disease that has the potential to cause irreversible liver injury and death from cirrhosis or liver cancer argues strongly in support of early diagnosis.

Wilson disease affects ~ 1 in 25,000 Americans and is caused by loss-of-function mutations in a gene encoding a transport protein that controls hepatic copper metabolism and biliary excretion. As with hemochromatosis, patients with Wilson disease slowly accumulate high levels of copper in tissues throughout the body, typically resulting in clinical presentation in late childhood or early adulthood, and, if untreated, eventually causing irreversible damage to liver, brain, and other tissues. Wilson disease can be treated using oral copper chelators such as penicillamine or trientine and/or oral zinc supplements. In some individuals with fulminant liver failure due to Wilson disease and in those with severe hepatic insufficiency due to the disease, liver transplantation may be necessary and life-saving. For these reasons, early diagnosis is critical in Wilson disease, as it can lead to prophylactic therapy that prevents the disease manifestations.

The porphyrias are a group of inherited diseases caused by defects in the synthesis and metabolism of heme, an iron-binding complex that plays a central role in reactions involving oxygen throughout the body. There are five main forms of porphyria that can affect the liver: acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), porphyria cutanea tarda (PCT), and erythropoietic protoporphyria (EPP). These are rare diseases, but they have dramatic and disabling clinical features, and they can lead to, or be associated with, end-stage liver disease. Clinical manifestations are due to the high levels of heme precursors (e.g., 5-delta-aminolevulinate, porphyrins) in blood or tissues that can cause acute neurological symptoms (as in AIP, HCP or VP), skin disease and photosensitivity (as in PCT and EPP), and chronic liver disease. Treatments for the porphyrias range from simple, highly effective therapies that reverse the disease manifestations (e.g., therapeutic phlebotomy for PCT) to more difficult and only partially effective means of ameliorating symptoms

(e.g., infusions of glucose and hematin, an oxidized form of heme, for AIP, HCP, VP and EPP). Some forms of porphyria respond to liver transplantation. Hepatocellular carcinoma risk is increased in most forms of porphyria, especially PCT.

Thus, the adult-onset genetic liver diseases are a diverse group of conditions that are uncommon or rare, but cause considerable burden and mortality to those affected.

Recent Research Advances

In the last 10 to 15 years, there have been substantial breakthroughs in the understanding of genetic liver diseases. The genes responsible for these diseases have been identified, cloned and sequenced, and the major mutations have been characterized. Importantly, the identification of these genes has also led to new insights into normal cell biology, frequently advancing knowledge about normal metabolism of iron, copper and porphyrins, as well as other acquired or metabolic diseases. At present, however, most of these gains in understanding have not led to significant improvements in diagnosis, treatment, or prevention and they require additional research efforts to close this translational gap.

Classical hereditary hemochromatosis was shown in 1996 to be due to mutations in the *HFE* gene, which codes for a cell surface protein belonging to the HLA gene family. Over 85% of Caucasian patients with typical hemochromatosis are homozygous for a single mutation in *HFE* (C282Y), and a lesser proportion are compound heterozygotes for this and another *HFE* mutation (H63D). Mice with deletions in the *Hfe* gene develop iron overload and a clinical phenotype similar to hemochromatosis. Further research has defined the tissue distribution of the HFE protein and documented its role in iron metabolism and cell signaling. Importantly, other genes coding for proteins involved in iron metabolism (e.g., divalent metal transporter 1, transferrin receptor-2, hephaestin, hemojuvelin and ferroportin-1) were found to be involved in hemochromatosis, and rarer forms of this disease have been linked to mutations in some of these genes. Recently, hepcidin—a small peptide secreted by hepatocytes—has been identified as a key sensor of tissue iron status and a modulator of iron absorption in the gut. Normally, hepcidin signals intestinal enterocytes to decrease levels of the proteins involved in iron absorption and macrophage iron release. Elucidation of the factors that regulate hepcidin will likely explain many features of body iron regulation.

A major clinical advance made possible by the discovery of *HFE* was the development of DNA tests for the C282Y and H63D mutations, which are now commercially available. Population-based surveys of serum iron levels and *HFE* mutations are now under way. These surveys will demonstrate the frequency of genetic disease in the general population and determine whether screening is appropriate to identify cases early, thereby enabling the prevention of liver, endocrine and heart disease due to hemochromatosis. They will also define the degree of penetrance of this gene mutation, i.e., what proportion of persons develops iron overload by a certain age.

Wilson disease has been linked to a copper transport protein, which is now referred to as ATP7B or Wilson ATPase. Multiple mutations in the gene coding for this ATPase have been found to be

associated with Wilson disease, and often more than one mutation contributes to the disease. There is some evidence for an association between genotype and phenotype in Wilson disease, with more severe disease observed in those patients with completely abrogated gene function. However, Wilson disease is one of the most diverse clinical syndromes associated with a single gene defect. The incomplete correlation between specific mutations and clinical manifestations remains unexplained and suggests an important role for modifier genes or unsuspected environmental factors. Knowledge of the Wilson ATPase has yet to be useful in early diagnosis, management or prevention of this disease, largely because less than half of cases can be identified by screening for the common Wilson ATPase mutations. Instead, diagnosis relies largely on copper and ceruloplasmin testing, as it did before discovery of the Wilson ATPase gene. Copper chelators and zinc supplements continue to be the treatments of choice. The function of the Wilson ATPase and the cellular mechanisms for the regulation of copper pathways involving this transporter remain poorly understood.

The genes that encode enzymes of the heme synthetic pathways, which are deficient in the hepatic porphyrias, have been identified, cloned and sequenced, and numerous mutations associated with porphyric phenotypes have been defined. In some situations, DNA analysis can be used for diagnosis and studies of structure-function relationships, as well as genotype-phenotype correlations. However, management of the porphyrias remains difficult. The recent observation of resolution of severe AIP after liver transplantation, demonstrating that the presence of a functional enzyme in the liver alone is sufficient to reverse the disease, has triggered new interest in gene therapy directed at the liver.

The case for an association of PCT with chronic hepatitis C and mild iron overload has been made through the use of newly developed diagnostic tests for hepatitis C and porphyria. However, the mechanisms by which hepatitis C and iron trigger clinical manifestations of PCT remain unclear. Finally, hepatobiliary disease in EPP is caused by the toxic effects of protoporphyrin, which is overproduced mainly in the bone marrow. This finding raises the prospect of bone marrow-based cell or gene therapy for severe cases of EPP.

Research Goals

The ultimate goals for research on genetic liver diseases are to develop practical and reliable means of screening and diagnosis, as well as to provide a means of control and prevention of their disease manifestations. Research on these genetic diseases will also provide new insights into normal vs. aberrant metabolism of iron, copper and porphyrins, which are likely to improve diagnosis and treatment of other diseases.

Although the genes of the major forms of genetic liver diseases have been identified, further work is warranted to elucidate the mechanisms of action of the enzymes, transporters, and signaling molecules that constitute the genetic defects in these diseases. It is particularly important to provide structure-function information about the mutated proteins and how they interact with other metabolic pathways. These details are ultimately important for identifying possible targets for intervention to alleviate the disease manifestations and prevent tissue damage.

Hemochromatosis: An important goal is to achieve a detailed definition of the normal molecular mechanisms of iron metabolism in humans (Matrix Cell B2). A specific research focus of importance is the role of HFE and its interactions with hepcidin. The pathways of iron metabolism are affected by, and are interwoven with, other cellular pathways and processes such as cytokine signaling, apoptosis, regeneration and repair. Knowledge of iron metabolism is likely to lead to insights into other diseases and their manifestations, such as alcoholic liver disease, nonalcoholic steatohepatitis (NASH), hepatitis C, and porphyria cutanea tarda (Matrix Cell B2). Not all cases of inherited forms of iron overload are due to the classical *HFE* mutations. This is particularly true among African Americans and Asian Americans, who rarely harbor the C282Y mutation yet have severe and unexplained forms of iron overload. An important goal is to identify the genetic causes of hemochromatosis in these ethnic groups and to improve screening and molecular diagnosis (Matrix Cell B3). The typical commercial assays for *HFE* mutations will not detect the lesser known causes of hereditary hemochromatosis, and, therefore, expert centers of excellence for DNA diagnostics and sequencing would help to more fully define the genetic causes of iron overload (Matrix Cell B1). For clinical management of hemochromatosis, it is important to define the frequencies of the gene mutations in the general population and how frequently these mutations result in iron overload and disease manifestations (Matrix Cell A1). Practical approaches to screening for iron overload and algorithms for molecular diagnosis would arise out of population studies and provide firm medical evidence for recommendations on screening in different populations at different ages (Matrix Cell B1). Patients with identical gene mutations often have markedly different clinical courses and expression of disease, suggesting an important role of gene modifiers in hemochromatosis; further definition of these modifiers would be helpful (Matrix Cell A1). Finally, management of both hereditary and acquired forms of iron overload would be greatly aided by an inexpensive, widely available, noninvasive means to assess total body and hepatic iron content (Matrix Cell C3). These noninvasive measures might be imaging tests or mathematical models based upon clinical features and results of blood tests and metabolic assays. The major challenge in hemochromatosis is the development of means to diagnose the disease early in persons who are likely to develop severe iron overload and tissue damage.

Wilson Disease: While the gene responsible for Wilson disease has been identified and the major mutations associated with disease have been characterized, the pathogenesis of copper overload and resultant cell injury have not been fully defined. An important goal for future research is to elucidate more completely the molecular mechanisms and pathways of intestinal absorption, hepatic metabolism and biliary secretion of copper (Matrix Cell A3). A major focus of this research should be on the role of Wilson ATPase and how mutations cause defects in excretion and disease manifestations (genotype-phenotype correlations). Improved tests for the mutations in Wilson ATPase and the availability of resources for testing of clinical materials would facilitate this research (Matrix Cell A1). Heterozygosity for Wilson ATPase may account for unusual manifestations of other liver diseases (Matrix Cell B1). More importantly, the great clinical heterogeneity of Wilson disease remains unexplained, warranting research efforts to define modifier genes in this condition that might explain why patients with the same Wilson ATPase mutations can present with widely different clinical manifestations (e.g., acute liver failure, cirrhosis, psychosis, neurological disease) (Matrix Cell C2). Therapy of Wilson disease is fairly straight-forward, but refinements in management would benefit patients. A major advance in the diagnosis and management of Wilson disease would be the development of a

means of assessing hepatic copper concentrations noninvasively, without dependence on liver biopsy (Matrix Cell C3). These resources would facilitate clinical studies of better approaches to management of this disease. Arguably, the most critical goal in Wilson disease research is the development of a practical and reliable means of metabolic diagnosis of Wilson disease that would serve not only as a diagnostic tool, but also as a way to screen the general population for this potentially fatal, but eminently treatable disease (Matrix Cell C1). A reliable screening test that could be applied to newborns or infants would allow for elimination of morbidity and mortality from this disease.

Porphyria: Although the relevant genes of the hepatic porphyrias have been identified, diagnosis and treatment of these diseases remain problematic. A central issue is the availability of molecular tests for the porphyrias, including sequencing capabilities to define the rarer mutations (Matrix Cell A1). A clearer definition of the metabolic pathways of heme synthesis and degradation would aid in defining the genotype-phenotype and structure-function relationships in the hepatic porphyrias. The molecular definition of these pathways would also help to define therapeutic targets for these diseases. The development of improved treatments for the acute crises of AIP is an important goal as hematin infusion therapy is expensive and only partially effective (Matrix Cell C2). Further elucidation of the pathophysiology of hepatic porphyrias, including the causes of the associated neuromuscular manifestations, the role of alcohol and estrogens in exacerbating porphyria symptoms, and roles of porphryrins in causing an increased susceptibility to HCC, would be helpful in this regard (Matrix Cells A2 and B3). Severe forms of porphyria represent excellent candidates for gene therapy. For instance, in EPP, the major source of the toxic protoporphyrin appears to be the bone marrow, and, therefore, bone marrow transplantation or replacement gene therapy may be a means of treatment. Similarly, in AIP, replacement of the defective enzyme in liver may reverse the systemic condition. Practical gene or stem cell therapies for AIP and EPP are important and represent high-risk goals for future research (Matrix Cell C3).

Steps to Achieve Research Goals

A major opportunity to help advance research in each of these genetic liver diseases is to create national resources for accurate genetic diagnosis. At present, the only commercially available DNA assays to identify gene mutations in these diseases are available for hemochromatosis to assess the classical C282Y and H63D mutations of *HFE*. However, even in the case of hemochromatosis, several other genes have been associated with rarer forms of this disease, and clearly new genes will be identified in the future associated with inherited forms of iron overload in African American, Hispanic, and Asian American patients. The availability of a national DNA diagnostic center with expertise in DNA sequencing and the various iron-related genes potentially linked to hemochromatosis would be enormously helpful. Perhaps even more helpful would be similar DNA diagnostic centers of excellence in Wilson disease and the porphyrias. At present, DNA testing for these diseases is done at a few sites, largely by prominent, NIH-funded basic researchers who take on such screening efforts at the expense of their basic research grants, which do not provide funds for such a resource that lacks a specific hypothesis-driven focus. Centers funded to provide a service, with state-of-the-art DNA sequencing capabilities, clinical laboratory expertise, and a dedication to characterize genes associated with these diseases, would

provide a nidus for further basic and clinical advances in these diseases. These DNA-testing centers could also provide a central database of patients' clinical information to help with genotype-phenotype correlations and to provide cohorts of well-characterized patients for clinical investigation and therapeutic trials of new agents or standardization of current therapies, such as in the optimal use of zinc supplements and copper-chelating agents in Wilson disease. These centers could also serve as a resource for patients, providing reliable information and fostering communication with other patients and researchers.

Continued research advances on genetic liver diseases will also require collaboration and coordination among NIH Institutes and other biomedical research funding agencies, such as the Department of Veterans Affairs, Centers for Disease Control and Prevention, and Food and Drug Administration, as well as leading private foundations. Specific *ad hoc* working groups on these diseases might facilitate these interactions and collaborations.

Matrix of Research Goals in Genetic Liver Disease

	Short-term Goals (0-3 years)	Intermediate-term Goals (4-6 years)	Long-term Goals (7-10 years)
High Risk	**A3. Fully elucidate the molecular mechanisms of intestinal absorption, hepatic metabolism and biliary excretion of copper.**	**B3. Identify the major genetic causes of inherited iron overload among African Americans, Asian Americans, and Hispanics.** **Define the molecular basis of the increase in HCC risk among persons with the porphyrias.**	**C3. Develop noninvasive means of accurately defining total body and hepatic iron and copper, either using imaging studies or mathematical models & serum levels of related molecules.** **Develop practical gene or stem cell therapy for AIP & EPP.**
Intermediate Risk	**A2. Elucidate the mechanism of neuromuscular manifestations of the acute hepatic porphyrias.** **Define roles of alcohol & estrogens in exacerbation of porphyrias.**	**B2. Fully define the normal molecular pathways of iron metabolism in humans with specific definition of the roles of HFE and hepcidin.** **Define the role of liver iron levels in the course of NASH, alcoholic liver disease, chronic hepatitis C & PCT.**	**C2. Define specific genetic modifiers of Wilson disease and porphyrias using animal models and clinical cohorts of patients.** **Develop an improved therapy for amelioration of acute crises in porphyria.**
Low Risk	**A1. More fully define the frequency of disease expression associated with *HFE* C282Y and define major modifying factors.** **Establish DNA evaluation centers of excellence for Wilson disease, the porphyrias and hemochromatosis.**	**B1. Develop and apply practical and accurate screening methods for identifying hemochromatosis before significant tissue injury has occurred.** **Define the role of heterozygosity for Wilson ATPase and *HFE* mutations in other liver diseases.**	**C1. Develop rapid metabolic screening test for Wilson disease that could also be applied to newborns or infants and assess test for efficacy and risk-benefit ratio.**